

## CLAIMS

1. An expression system for producing a B subunit of a cholera toxin (CTB) wherein the expression system comprises:
  - 5 (c) a *Vibrio cholerae* host cell lacking the functionality of a *thyA* gene; and
  - (d) an expression vector less than 5kb in size comprising a functional *thyA* gene and a CTB gene which is substantially free of the flanking sequences immediately contiguous by the 5' and 3' end of the CTB gene in the naturally occurring genome of the host cell from which the CTB gene is derived.
- 10 2. The expression system according to claim 1 wherein the host cell lacks the functionality of a CTA gene.
3. The expression system according to claim 1 or 2 wherein the expression vector is about  
15 3kb in size.
4. The expression system according to any one of claims 1 -3 wherein the expression vector comprises an *E. coli thyA* gene.
- 20 5. The expression system according to any one of claims 1 -4 wherein the expression vector has the nucleotide sequence presented in SEQ ID NO:1.
6. The expression system according to any one of claims 1-5 wherein the expression vector further comprises at least one further nucleotide sequence encoding a heterologous protein.  
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7. The expression system according to claim 6 wherein the further nucleotide sequence encodes a non-toxic component or form of the heat labile *E. coli* enterotoxin LT, preferably the non-toxic component of LT is the B subunit of a (LTB) or a fragment thereof.
- 30 8. A method of producing CTB wherein the method comprises:  
transforming a *Vibrio cholerae* host cell lacking the functionality of a *thyA* gene with an expression vector less than 5kb in size comprising a functional *thyA* gene and a CTB gene which is substantially free of the flanking sequences immediately contiguous by the 5' and 3' end of the CTB gene in the naturally occurring genome of the host cell from  
35 which the CTB gene is derived,  
and

culturing the transformed *V. cholerae* host cell under conditions which permit production of the CTB.

9. The method of claim 8 wherein the method further comprises isolating and/or purifying the  
5 CTB from the host cell.

10. An isolated nucleic acid construct which comprises a *thyA* gene and a CTB gene which is substantially free of the flanking sequences immediately contiguous by the 5' and 3' end of the CTB gene in the naturally occurring genome of the host cell from which the CTB gene is  
10 derived, and which nucleic acid construct is less than 5kb in size.

11. The nucleic acid construct according to claim 10 wherein the nucleic acid construct is about 3kb in size.

12. The nucleic acid construct according to claim 10 or 11, wherein the nucleic acid construct  
15 is a plasmid.

13. The nucleic acid construct according to claim 12, wherein the plasmid is pMT-ctxB*thyA*-2 characterised by a restriction endonuclease map as shown in Figure 13.

14. The nucleic acid construct according to claim 12, wherein the plasmid has the nucleotide  
20 sequence SEQ ID NO: 1.

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